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Cell Movements during Gastrulation of Starfish Larvae

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Abstract. Archenteron formation was monitored by measurement of cellular volume, injection of tracer enzyme, and vital staining. The cellular volume of the whole embryo did not change significantly from the start of gastrulation to the beginning of the mesenchyme-migration stage; the archenteron increased from about 10–20% during these stages. Tracer injection revealed that the boundary between the progenies of the veg1 and veg2 blastomeres of 32-cell-stage embryos was in the outer layer at the early gastrula stage, and at the rear end of the stomach at the bipinnaria stage. These results demonstrate a migration of cells from the outer layer to the archenteron wall during starfish gastrulation. Vital staining marks around the blastopore showed that the presumptive esophagus, stomach, and intestine area were added to the archenteron at the start of gastrulation, during the early to late gastrula stage, and thereafter, respectively. Tracer injection also indicated that the presumptive zone of the cardiac sphincter was twisted about 180° clockwise around the axis of the archenteron after the late gastrula stage, dragging the cells in the presumptive zone of the esophagus and stomach.

Introduction

Gastrulation is a striking event in the early development of echinoderms. It involves a dynamic morphological change from a monolayered to a multilayered embryo, accompanied by differentiation of the mesendoderm from the ectoderm.

Starfish embryos are good materials for investigation of the mechanism of gastrulation because both oocytes and embryos of starfish have markers for the presumptive site of archenteron formation (Schroeder, 1985; Kuraishi and Osanai, 1989) and, because the oocytes, embryos, and blastomeres are larger than those of sea urchins. This provides an advantage in microsurgery. In addition, both oocyte fragments and blastomeres derived from the animal hemisphere have little capacity for archenteron formation (Maruyama and Shinoda, 1990; Zhang *et al.*, 1990), unlike those of sea urchins (Henry *et al.*, 1989; Khaner and Wilt, 1990). They would be good recipients for implantation experiments to investigate cytoplasmic determinants responsible for archenteron formation.

Despite such advantages, little is known about the morphogenetic movements of archenteron formation in normal starfish larvae. Thus, we traced the movement of cells into the archenteron wall by measuring the volume of the archenteron wall, tracer enzyme injection, and vital staining.

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Materials and Methods

Materials

Adult individuals of *Asterina pectinifera* were collected during their breeding season at Asamushi, Aomori prefecture, and at Hashirimizu, Kanagawa prefecture (Japan). Gametes were prepared as described elsewhere (Kuraishi and Osanai, 1988). Oocyte maturation was induced by treatment with 1 μm 1-methyl adenine (1-MeAde). The oocytes were fertilized about 45 min after the start of 1-MeAde treatment. The fertilized eggs were washed with filtered seawater and allowed to develop at about 19°C.

Measurement of cellular volume in gastrulae

The cellular volume of the archenteron and the outer layer was calculated based on the practically rotationally symmetrical shape around the animal-vegetal axis. The larvae were photographed through a plane including the animal-vegetal axis. The boundary between the archenteron and outer layer was determined tentatively in the plane as a line running parallel to the axis and passing the most posterior point of the basal surface of the

embryonic wall. An imaging, two-dimensional coordinate system was prepared on the photographic print where the animal pole and animal-vegetal axis were defined as the origin and x-axis, respectively. Then the grids that were perpendicular to the x-axis were settled at every micrometer along the x-axis. The shape of the archenteron and outer layer was recorded by establishing the coordinates where these outlines crossed the grids, using an electronic digitizer. The volume was measured by calculating that of the solid of revolution obtained by rotating the shape around the animal-vegetal axis. In order to photograph the gastrulae through the mid-sagittal optical plane with minimum deformation resulting from immobilization, ciliary movement of the gastrulae was inhibited with 100 m*M* sodium azide in 80% filtered seawater, and the gastrulae were transferred to a wedge-shaped "egg holder" (Kishimoto, 1986). The holder was placed narrow side down for a few minutes, to allow most of the gastrulae to settle in the wedge with their lateral side against the wall. Then optical sections of the gastrulae including the anteroposterior axis were photographed. The concentration of sodium azide required for inhibition of ciliary movement was much higher than that for the sea urchin, *Hemicentrotus pulcherrimus* (Kominami, 1988). However, no unfavorable influence was observed after this treatment, providing the duration was less than 15 min.

Intracellular injection of horseradish peroxidase

A tracer enzyme, horseradish peroxidase (HRP), was injected into blastomeres iontophoretically following the procedure of Nishida (1987). The equipment used for micro-manipulation was set up according to Maruyama *et al.* (1986). The electrodes were made by pulling out glass capillary tubes with inner fibers (GD-1, Narishige Sci. Inst. Lab.) using a microelectrode puller (PG-1, Narishige Sci. Inst. Lab.). They were filled with 2% HRP in 0.2 *M* KCl at the tip, and then backfilled with 0.2 *M* KCl by sandwiching a small amount of silicone oil. The resistance of each electrode was adjusted to 15–30 MΩ by breaking the tip. HRP was introduced into the blastomere with a positive current of 10–15 nA for 30–90 s. The embryos were then transferred separately into individual holes of a 24-hole culture plate.

To examine the distribution of descendant cells of the injected blastomere, they were fixed in 1% glutaraldehyde in filtered seawater overnight at 4°C. The fixed specimens were also stained histochemically according to Nishida (1987), and observed as whole mounts using light microscopy.

Vital staining of larvae

Gastrulae were suspended in 0.05% Nile blue sulfate in filtered seawater for 30–60 s and washed thoroughly with filtered seawater to mark the outer layer. This treatment only stained deeply the Nile blue-positive granules (Kuraishi and Osanai, 1989) of cells in the outer layer.

Larvae were first held in an egg holder to individually mark the blastopore lip and the archenteron of the gastrulae. A fine glass capillary with a 1 μm diameter tip filled with 1% Nile blue sulfate in distilled water was pushed against the area to be stained and the Nile blue solution was ejected gradually for about 30 s by pressure. Because the position of the stained region differed among the larvae, the stained larvae were photographed and reared individually in each hole of the 24-well culture plate. For observation and microphotography of the swimming larvae at later stages, their ciliary movement was inhibited by 100 mM sodium azide in 80% filtered seawater and then they were mounted in an egg holder. The orientation of the larvae was adjusted by micromanipulation.

Results

Morphological aspects of gastrulation

The larvae started gastrulation about 17–18 h after onset of 1-MeAde treatment. Hereafter, the developmental time referred to represents the time after 1-MeAde treatment. At the beginning of gastrulation, the vegetal plate covering about half of the larva's diameter invaginated at the posterior end (vegetal pole) of the larva (Fig. 1A). The gastrula stage was divided into five substages according to Dan-Sohkawa *et al.* (1986).

The early gastrula stage extended from the start of gastrulation to 22 h, during which the blastopore gradually decreased in diameter (Fig. 1A, B). At the end of this stage, the length of the archenteron had reached about ⅓ that of the whole larva.

The mesenchyme differentiation stage extended from 22–27 h. The body of the larva elongated along the anteroposterior axis, and the length of the archenteron reached half that of the whole larva by the end of this stage. The tip of the archenteron appeared spherical and swelled to ½ of the larva's diameter. The cell layer of this region became thinner than that of the other parts of the embryo. The rest part of the archenteron appeared cylindrical, with an outer diameter about ¼ that of the outer layer (Fig. 1C).

The mesenchyme migration stage extended from 27 to 35 h. The appearance of the larvae was almost the same as that at the mesenchyme differentiation stage, except for the presence of mesenchyme cells in the blastocoel, having ingressed from the tip of the archenteron.

The late gastrula stage extended from 35 to 40 h. The mesenchyme cells spread all over the blastocoel, and the

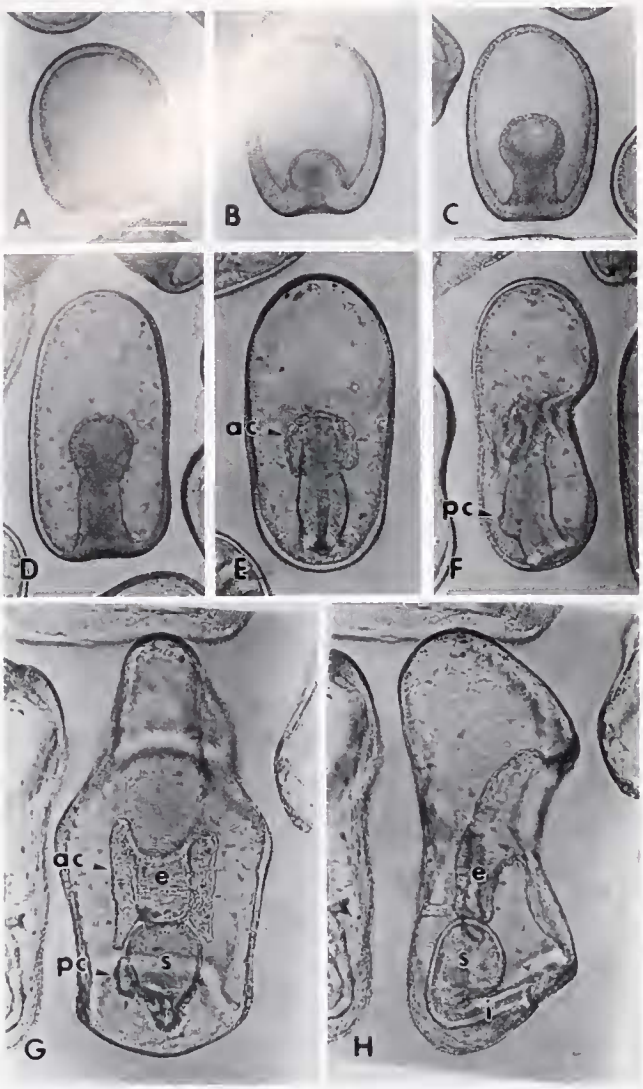

Figure 1. Normal larvae of *Asterina pectinifera* (A) start of gastrulation; (B) early gastrula stage; (C) mesenchyme-differentiation stage; (D) late gastrula stage; (E, F) mouth-formation stage; and (G, H) bipinnaria stage. (E, G) Observed from dorsal side. (F, H) Observed from right side. ac = anterior coelom, pc = posterior coelom, e = esophagus, s = stomach, i = intestine. Scale bar = 100 μm.

outer layer became flattened slightly in a dorso-ventral direction (Fig. 1D).

Mouth-formation stage extended from 40 to 48 h, during which time the body became much more flattened in a dorso-ventral direction. The blastopore decreased in diameter and moved to the ventral side (Fig. 1F). A pair of coelomic rudiments projected to the lateral side from the tip of the archenteron (Fig. 1E). The outer diameter of the posterior ¼ of the rest of the archenteron started to increase gradually. A rudiment of the left posterior coelom projected into the blastocoel from the dorsal wall of this widening region and began to migrate to the left side (Fig. 1F). The archenteron began to bend toward the ventral side, where invagination of the stomodaeum had already begun.

Larvae reached the early bipinnaria stage at about 72 h. The digestive tract had differentiated into the esophagus, stomach, and intestine. The boundary between the esophagus and stomach was sharply constricted by the cardiac sphincter. The boundary between the stomach and intestine was not so obviously constricted by the pyloric sphincter compared to that of sea urchins. Coeloms existed on both sides of the esophagus and by the left side of the stomach (Fig. 1G).

Cellular volume of archenteron

In sea urchins, translocation of cells from the outer layer into the archenteron wall occurs only in the first few hours of gastrulation (Horstadius, 1973; Hardin, 1989; Burke *et al.*, 1991). To clarify whether this also occurs in

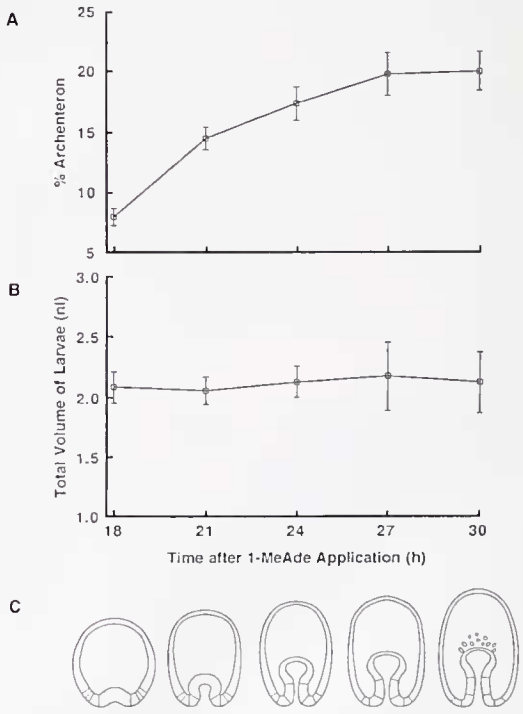

Figure 2. (A, B) Change in the total volume of the embryonic wall of normal gastrulae (B) and relative volume of the archenteron wall against the total (A) in a typical batch of *Asterina pectinifera* (C) Outlines of typical gastrulae at the start of gastrulation (18 h), early gastrula stage (21 h), mesenchyme-differentiation stage (24 h, 27 h), and mesenchyme-migration stage (30 h) shown with calculated 10, 15, 20, and 25% relative volume lines. The volumes were measured in more than 10 individuals for each data point in A and B. The error bar represents the 99% confidence interval. The volume of the mesenchyme cells was neglected in volume measurement at 30 h.

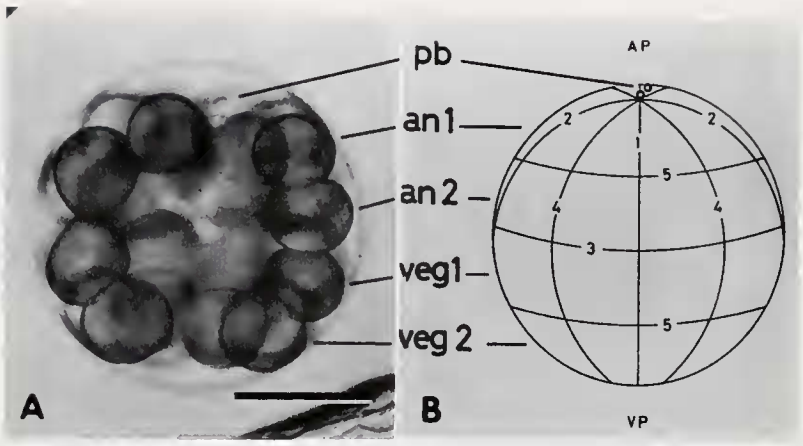

Figure 3. A 32-cell-stage embryo of *Asterina pectinifera* (A) and a schematic illustration showing the position of cleavage planes up to the 5th cleavage (B). AP = animal pole, VP = vegetal pole, pb = polar bodies, 1 = first cleavage plane, 2 = second cleavage plane, 3 = third cleavage plane, 4 = fourth cleavage plane, 5 = fifth cleavage plane. Though each cleavage divides the blastomere almost equally, the axis of animal-vegetal polarity is detectable by the position of the polar bodies. Scale bar = 100 μm.

starfish gastrulation, the volume of the embryonic wall in both the archenteron and the outer layer was measured. Since the volume was calculated assuming that the body of the larva was rotationally symmetrical around the antero-posterior axis, only larvae younger than the mesenchyme migration stage were used.

The relative volume of the vegetal plate, which invaginated at the start of gastrulation, ranged from 8 to 10% of the whole volume of the embryonic wall in the three batches used (Fig. 2A). The volume ratio of the wall of the archenteron increased to about 15% by the end of the early gastrula stage, and to about 20% by the end of the mesenchyme differentiation stage (Fig. 2A). In spite of the increase in the relative volume of the archenteron wall, the cellular volume of the whole embryo did not change significantly, and was almost identical to that of immature oocytes. This suggests that translocation of cells into the archenteron continues at least until the mesenchyme differentiation stage.

The areas having volume ratios of 10, 15, 20, and 25% from the tip of the archenteron were also calculated in each of the larvae used for volume measurement. Figure 2C shows outlines of typical specimens in the batch used to plot Figure 2A and B, with the 10, 15, 20, and 25% lines indicated.

Intracellular injection of HRP

In sea urchins, descendants of veg2 cells invaginate as the vegetal plate during the primary phase of gastrulation and form most of the archenteron (Horstadius, 1973; Davidson, 1989). To clarify if this occurs in starfish gastrulae, a tracer enzyme, HRP, was injected into cells in the tiers occupying the vegetal or subequatorial quarter of the embryo. The labeling pattern was then observed at the early gastrula and bipinnaria stages. A typical embryo of *A. pectinifera* showed almost equal orthoradial cleavage (Fig. 3B). At the 32-cell stage, the embryo consisted of four tiers, each containing eight cells. These tiers were named an1, an2, veg1, and veg2 from the animal pole to the vegetal pole (Fig. 3A). HRP was injected into one of the veg1 or the veg2 blastomeres at this stage. When one of the veg1 blastomeres was injected with HRP, the labeled cells were distributed only in the posterolateral region of the outer layer at the early gastrula stage (Fig. 4A, B). At the bipinnaria stage, the labeled cells were distributed not only in the posterior part of the ectoderm but also in the intestine and the rear end of the stomach (Fig. 4D). However, when one of the veg2 blastomeres was injected, the labeled cells were distributed from the tip of the archenteron to the posterior end of the outer layer at the early gastrula stage (Fig. 4C), and from the esophagus to the rear end of the stomach at the bipinnaria stage (Fig. 4E). This shows that the boundary area between veg1 and veg2 was added to the archenteron after the early gastrula stage.

The band of labeled cells was twisted torsionally in the esophagus and in the stomach (Fig. 4E). The results of careful observation of this aspect are described later.

Vital staining with Nile blue

To observe the movement of involuting cells during the gastrula stage more precisely, cells in either the outer layer or the archenteron were vitally stained with Nile

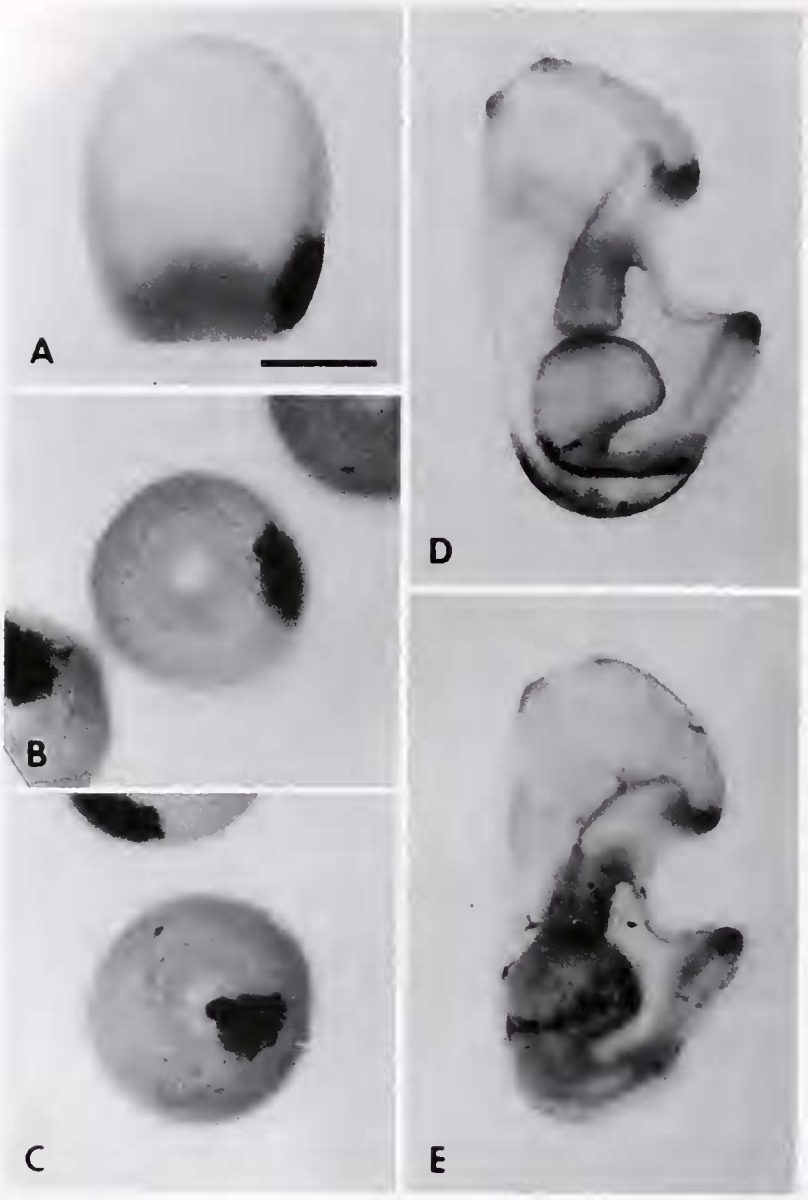

Figure 4. Larvae of *Asterina pectinifera* following injection of HRP into one of the veg1 blastomeres (A, B, D) and veg2 blastomeres (C, E) at the 32-cell stage, observed at the early gastrula stage (A–C), and the bipinnaria stage (D, E). A, D, E shown in lateral view, B in rear view of the same larva as that in A1, and C in rear view. The boundary between the labeled regions is located in the outer layer at the early gastrula stage (A–C), but at the rear end of the stomach at the bipinnaria stage (D, E). Scale bar = 100 μm.

blue. When the outer layer was stained at the early gastrula stage (Fig. 5A2), the cylindrical part of the archenteron consisted of stained cells from the mesenchyme differentiation stage to the late gastrula stage (Fig. 5B2, C2). After the mouth-formation stage, the outer diameter of the stained region of the archenteron increased (Fig. 5D2). At the bipinnaria stage, the stomach, the intestine, and the left posterior coelom consisted of stained cells (Fig. 5E2). In bipinnariae which were stained at the mesenchyme differentiation stage, the posterior part of the stomach and the intestine consisted of stained cells (Fig. 6A1, A2). When the outer layer was stained at the late gastrula stage, the area of Nile blue was observed all over the intestine other than the ectoderm (Fig. 6B1, B2). When the posterior end of the archenteron near the blastopore or the blastopore lip was stained as a control to check

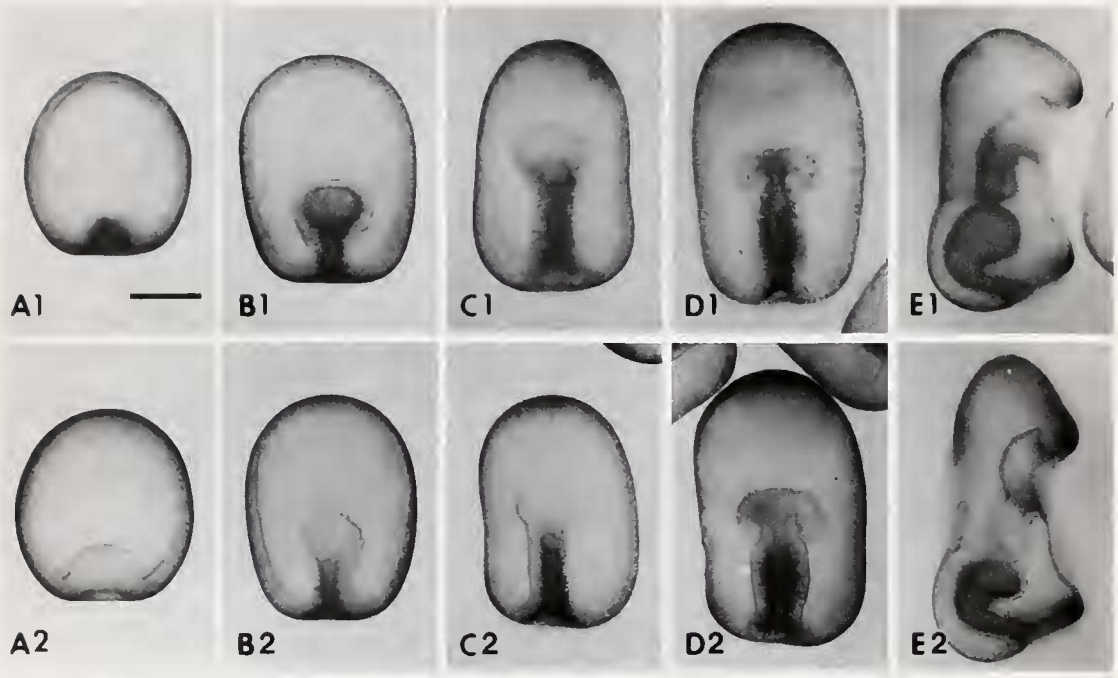

Figure 5. Larvae of *Asterina pectinifera* stained with Nile blue at the blastula stage (1) and at the early gastrula stage (2). (A) early gastrula stage, (B) mesenchyme-differentiation stage, (C) late gastrula stage, (D) mouth-formation stage, (E) bipinnariae stage. When larvae were stained at the early gastrula stage, stained Nile blue-positive granules were not observed in the archenteron (A2), tip of the archenteron (B2–D2), or the esophagus (E2). Scale bar = 100 μm. Since the larvae are flattened due to pressure from the coverslips in 1–4, they appear larger than usual.

dispersal of the dye, unstained cells became detectable behind the stained region in the archenteron in the later stages (Fig. 7).

The boundary between the stained and unstained regions in larvae that were stained at the early gastrula stage almost coincided with the position of the 10% line in Figure 2C at both the early gastrula and mesenchyme differentiation stages (Fig. 5A2, B2). This confirms that vital staining is a reliable method for monitoring cellular movement during the gastrulation process.

Lateral movement of archenteron cells

When HRP was injected into one of the veg2 blastomeres in a 32-cell-stage embryo, the band of labeled cells appeared twisted torsionally in the digestive tract at the bipinnaria stage (Fig. 4D, E). To examine this twisting more precisely, one of the blastomeres in the vegetal hemisphere of an 8- or 16-cell-stage embryo was injected with HRP and the distribution of labeled cells was observed at the bipinnaria stage. Figure 8 shows four complementary patterns in which left ventral (A), left dorsal (B), right dorsal (C), and right ventral (D) regions in the posterior part of the ectoderm were labeled. In all cases, the band of labeled cells was parallel to the axis of the digestive tract from the anus to the posterior end of the stomach, sharing the same orientation as the ectoderm relative to the dorso-ventral axis. However, the band was twisted torsionally about 180° clockwise from the posterior end of the stomach to the cardiac sphincter, and about the same degree counterclockwise from the cardiac sphincter to the stomodaeum.

To determine when the digestive tract (or the archenteron) became twisted, the distribution of labeled cells was also observed at earlier stages. From the early gastrula stage to the mesenchyme differentiation stage, the bands of the labeled cells were not twisted (Fig. 9A). At the late gastrula stage, the band was twisted slightly clockwise at the anterior part of the cylindrical region of the archenteron and counterclockwise at the posterior half of the inflated region (Fig. 9B). At the mouth formation stage, the band was twisted much more evidently (Fig. 9C). These results show that the presumptive region of the cardiac sphincter rotated about 180° clockwise around the longitudinal axis of the archenteron, dragging the presumptive zone of the esophagus and stomach, after the mesenchyme-differentiation stage.

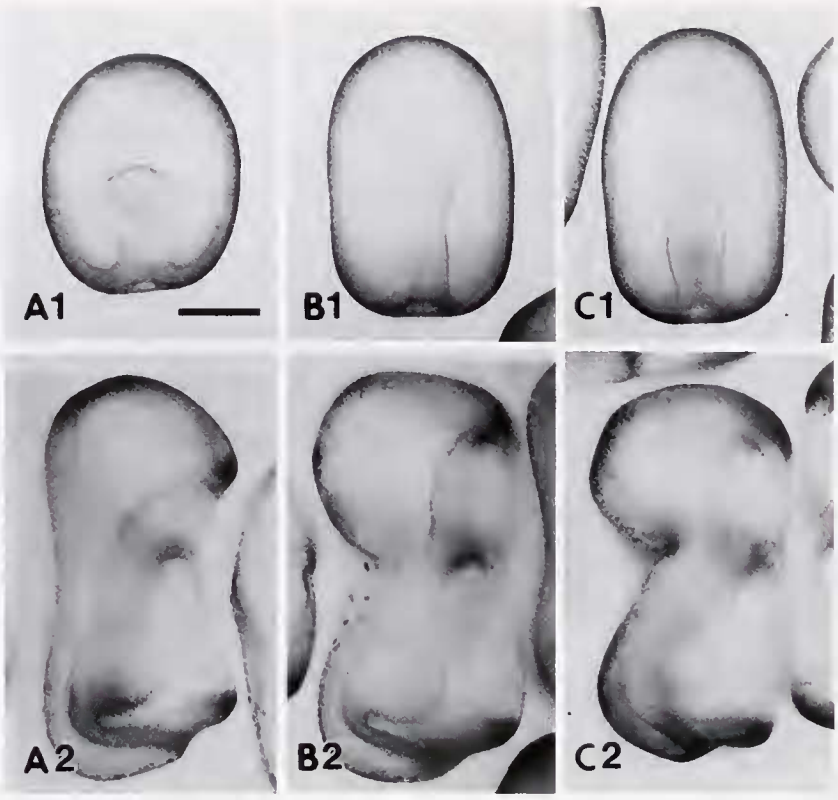

Figure 6. Larvae of *Asterina pectinifera* stained with Nile blue at the mesenchyme-differentiation stage (A), the late gastrula stage (B), and the mouth-formation stage (C), observed just after staining (1) and at the bipinnaria stage (2). The whole archenteron (A–C), esophagus and anterior half of the stomach (A2–C2), posterior half of the stomach (B2, C2), and anterior half of the intestine (C2) are constructed of unstained cells. Scale bar = 100 μm.

When the left dorsal side of the ectoderm was labeled, labeled cells distributed only in the anterior region in the left posterior coelom (Fig. 8B2). In contrast, when the right dorsal side of the ectoderm was labeled, the labeled cells distributed only in the posterior region in the left posterior coelom (Fig. 8C2).

Discussion

The present study demonstrates that in gastrulae of *A. pectinifera*, cells at the rear end of the outer layer are added to the archenteron throughout the gastrula stage, constructing the posterior part of the archenteron. The volume ratio of the archenteron was 8–10% at the start of gastrulation, increasing to 15% at the early gastrula stage and reaching 20% at the start of the mesenchyme migration stage. Kominami (1984) showed that the relative volume of the archenteron wall ranges from 20 to 25% at the early to mid gastrula stage (= mesenchyme migration stage in this study) in *A. pectinifera*. This result is almost consistent with our result obtained from 30-h embryos. He considered that the vegetal quarter of the embryos would differentiate into the mesendoderm, provided that the amount of cellular material in the archenteron did not increase afterward. However, the result of vital staining shows that translocation of cells into the archenteron wall continued thereafter.

Evidence from HRP-injection in the present study indicate that the coeloms, mesenchyme cells, esophagus, and the stomach are formed of veg2 descendants. The result of the vital staining shows that the presumptive area of these organs are added to the archenteron from the start of gastrulation to the late gastrula stage. Since cleavage in *A. pectinifera* is almost equal, the volume of each tier is about 25% of the whole volume of the embryo at the 32-cell stage. Thus, the increase in the volume of the archenteron during the mesenchyme-migration and late-gastrula stage is calculated to be about 5% (25 − 20%) of the whole volume of the larvae. The intestine is mainly formed of veg1 descendants (Fig. 4D), which are added to the archenteron after the late gastrula stage (Fig. 6C). The length and the outer and inner diameter of the

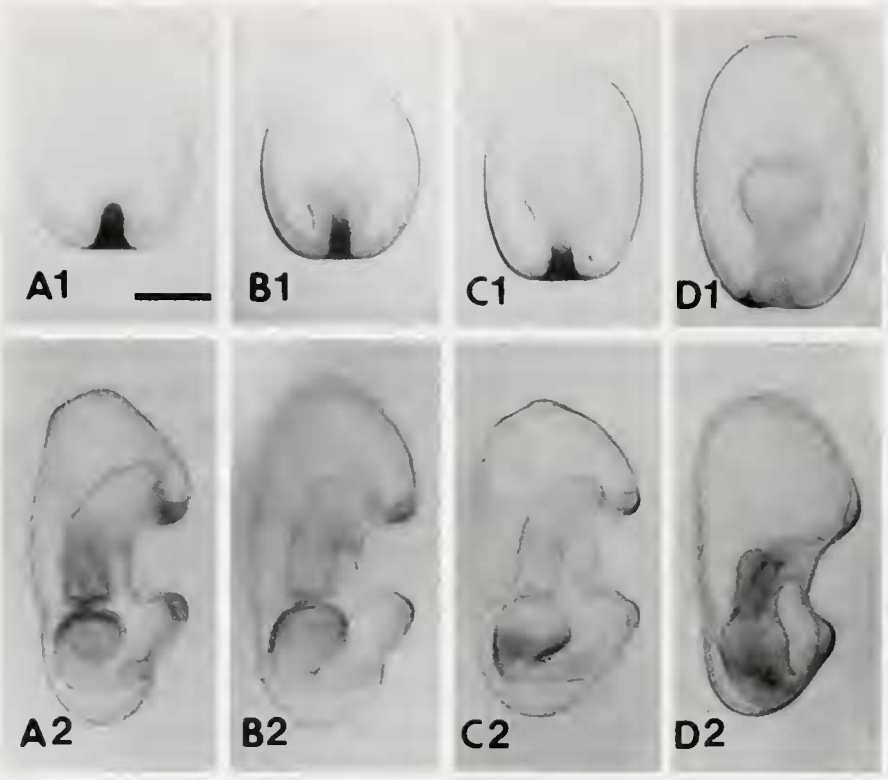

Figure 7. Larvae of *Asterina pectinifera* in which the posterior part of the archenteron near the blastopore (A–C) or the blastopore lip (D) was stained with Nile blue. Row 1 shows larvae just after staining. (A) early gastrula stage, (B) mesenchyme differentiation stage, (C) late gastrula stage, and (D) mesenchyme migration stage. Row 2 shows the same larvae as those in row 1 observed at the bipinnaria stage (A–C) or at the end of the mouth formation stage (D). Scale bar = 100 μm.

intestine of the bipinnaria in Figure 2H are 150 μm, 30 μm, and 10 μm, respectively. The volume is calculated to be about 0.1 nl (about 5% of the whole volume of the larvae before the mesenchyme-migration stage), assuming that it is a simple tube. Thus, volume increase of the archenteron wall continues after the mesenchyme-migration stage, though the rate decreases considerably.

In sea urchins, cellular materials are also added to the archenteron wall from the outer layer during the primary phase of gastrulation (Ettensohn, 1984; Burke *et al.*, 1991), while the apico-basal length of the cells around the vegetal pole decreased only slightly. These facts indicate that the buckling of the cell sheet is not the result of cell rounding, as suggested by Gustafson and Wolpert (1963, 1967) (Ettensohn, 1984). Our results shows that this is also the case in starfish.

In sea urchin embryos, however, translocation of cells from the outer layer to the archenteron wall only lasts for the first few hours of gastrulation (Hardin, 1989; Burke *et al.*, 1991) and the active cell rearrangement is proposed to be the major mechanism for the archenteron elongation thereafter (secondary phase of gastrulation) (Ettensohn, 1985; Hardin and Cheng, 1986; Hardin, 1989). Hardin (1989) showed that the cell number and the volume of the archenteron did not change significantly during the secondary phase of gastrulation, though the definition of the archenteron area is not clearly demonstrated in his report. The volume of the boundary region is relatively large because the thickness of the larval wall and the distance from the axis of symmetry is greater than in other parts of the archenteron. Cell number and calculated volume would thus vary widely depending on where the boundary is determined. It might be possible that different definition in the archenteron area lead to the different conclusion. However, the evidences from the vital staining at the rear end of the larvae also indicate that the cell addition from the outer layer occurs only in starfish in later stages of gastrulation (Hardin, 1984; Burke *et al.*, 1991; Figs. 6, 7). On the other hand, the band of HRP-labeled cells in the gastrula appeared narrow in later stages (Fig. 9) suggesting that rearrangement of cells may also be the mechanism of archenteron elongation in starfish. The change in the mechanism of gastrulation appears to progress rather more gradually in starfish than sea urchins.

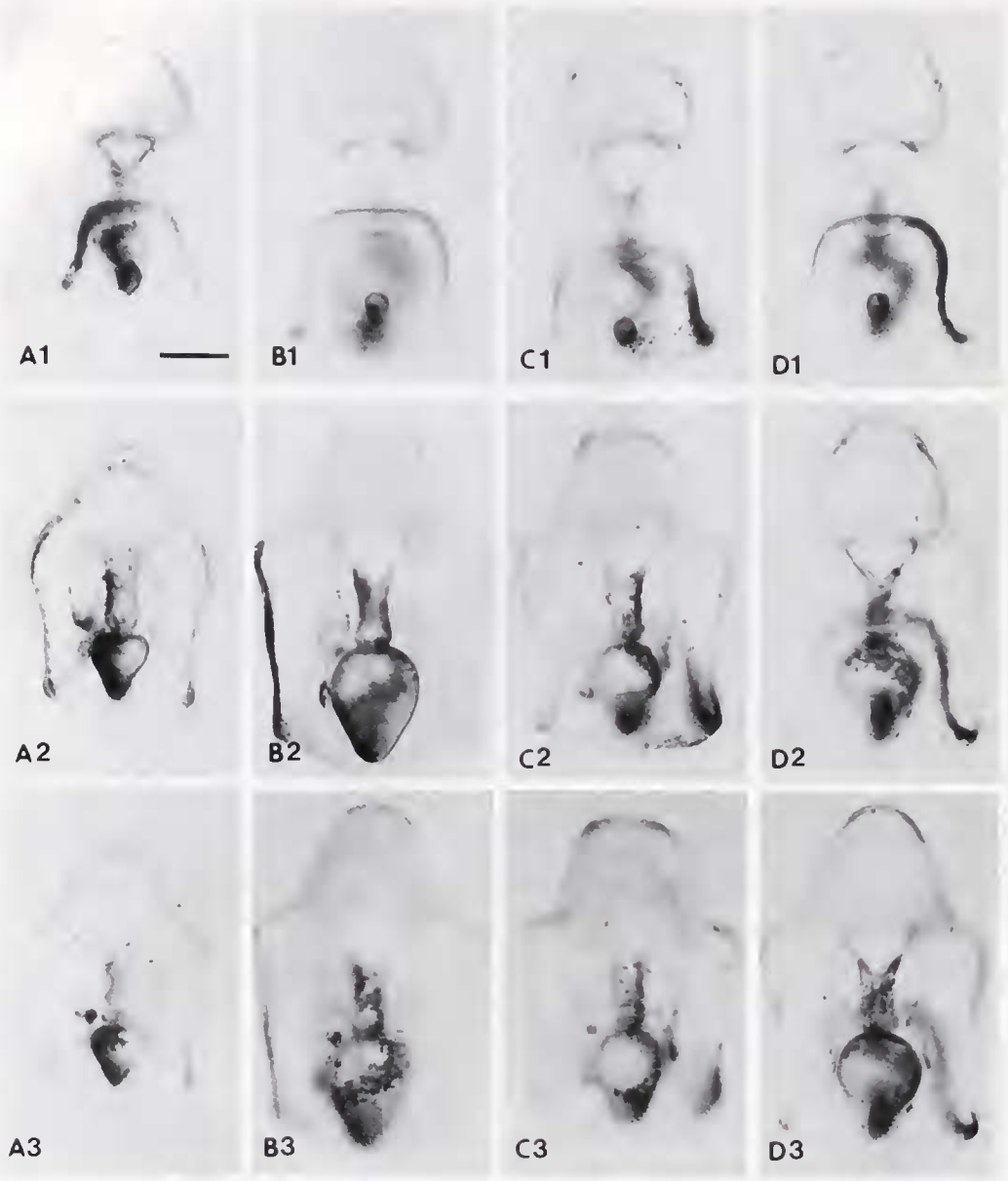

Figure 8. Bipinnariae in which one of the vegetal-half blastomeres was injected with HRP at the 8-cell stage. The left ventral (A), left dorsal (B), right dorsal (C), and right ventral (D) parts of the posterior ectoderm and part of the digestive tract are stained, respectively. Scale bar = 100 μm. All photographs are printed to allow observation from the dorsal side. A1–D1 are focused at the anus; C2 and D2 are focused at the ventral surface of the stomach; A2, B2, and D3 are focused at the axis of the esophagus; and A3, B3, and C3 are focused at the dorsal surface of the stomach. The band of labeled cells is twisted clockwise in the stomach, and counterclockwise in the esophagus in all cases. The anterior and posterior halves of the left posterior coelom are labeled in B2 and C2, respectively.

The movements of mesendoderm cells in larvae of *A. pectinifera* are shown in Figure 10. The cellular volume of the vegetal plate which invaginated at the start of gastrulation is 8–10% of the total volume of the embryo. Later, this region becomes the swelled region at the tip of the archenteron and sheds mesenchyme cells into the blastocoel. The anterior half of this region begins to project laterally at the mouth-formation stage, and becomes the anterior coelom of the bipinnaria. The posterior half of this region differentiates into the esophagus. The cells in the presumptive stomach and left posterior coelom area are added to the archenteron from the early gastrula stage

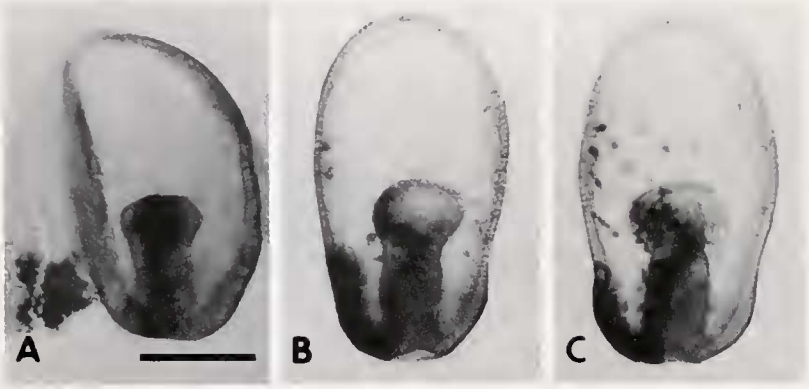

Figure 9. Gastrulae of *Asterina pectinifera* in which one of the vegetal blastomeres was injected with HRP at the 8- or 16-cell stage. (A) mesenchyme-differentiation stage, (B) late gastrula stage, and (C) mouth-formation stage. The front ectoderm is removed in (A) to show the labeled cells in the archenteron more clearly. The band of labeled cells in the archenteron is twisted in (B) and (C), but not in (A). Scale bar = 100 μm.

to the end of the late gastrula stage. The coeloms, esophagus, and stomach are formed mainly from veg2–descendants. Since cleavage in *A. pectinifera* embryos is almost equal, the volume ratio of each tier may be about 25% of the whole volume. Thus, the volume ratio of the stomach is calculated to be about 15% (25 − 10%). The presumptive intestine area is generally originated from the veg1 tier and added to the archenteron after the late gastrula stage. The volume of this area is calculated to be about 5% of that of the whole larvae. Thus, the total volume of the mesendoderm finally reaches about 30% of the whole volume of the larvae before the mesenchyme-migration stage.

After the late gastrula stage, the cells in the presumptive zone of the cardiac sphincter began to migrate laterally clockwise on the archenteron wall, dragging the cells in the presumptive esophagus and stomach zone. The rest of the archenteron maintained its original orientation in relation to the dorso-ventral axis. The significance and the mechanism of this torsional twisting of the archenteron was not examined in this study. However, it is possible that this twisting may be related to the mechanism of the constriction of this region, since the twisting occurs at the same area and about the same time as the constriction.

The labeling pattern of the left posterior coelom in HRP-injected larvae showed that the cells in the anterior and the posterior region of this vesicle originated from the left dorsal and right dorsal region, respectively. The rudiment of the left posterior coelom projected into the

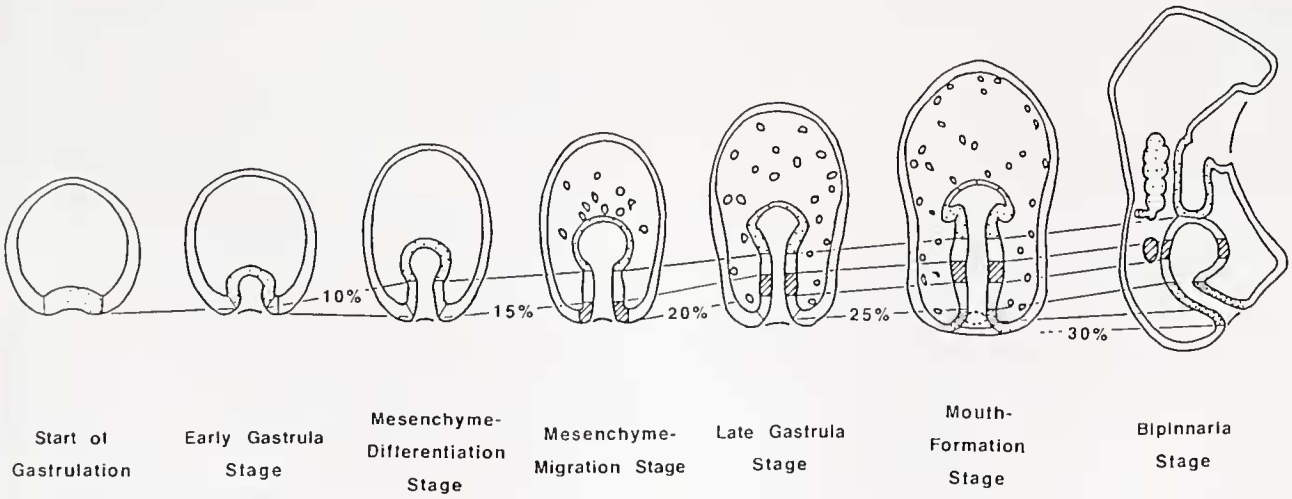

Figure 10. Schematic illustration of the gastrulation process of *Asterina pectinifera*. Lines between each of the profiles link the points that are involuted at the same point of development.

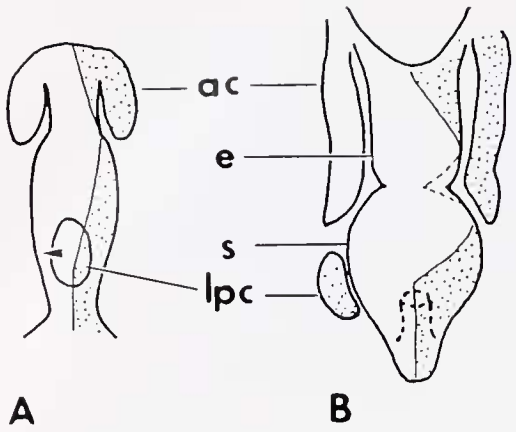

Figure 11. Schematic illustration of formation of the left posterior coelom. (A) mouth-formation stage. (B) bipinnaria stage. ac = anterior ceoloms, e = esophagus, s = stomach, lpc = left posterior coelom. Dotted region consists of cells from the original right side.

blastocoel from the dorsal side of the archenteron at the mouth formation stage, when the twisting had already been initiated. The anterior region of this rudiment projected from the twisting area where the original left dorsal cells had migrated, while the posterior region consisted of cells of the mid dorsal region. The rudiment then migrates to the left side during the mouth formation stage. As a result, the anterior and posterior halves of the left posterior coelom consist of cells from different longitudes (Fig. 11).

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